...partment of Agriculture Agricultural Research Service December 1990

Agricultural Research

Here comes trouble. But how much?

Story on page 4.

FORUM

Norman F. Cheville, Distinguished Scientist of 1990

For his pioneering work using the electron microscope to advance the understanding of animal disease processes, the Agricultural Research Service has named Norman F. Cheville Distinguished Scientist of the Year 1990.

Through studies of the changes in cellular structure caused by viruses and bacteria, he set out to demonstrate the ways in which these disease-causing agents develop inside animal cells. He and collaborators zeroed in on several viruses of poultry in the 1970's, clarifying the intracellular life cycles of the viruses of fowlpox, Newcastle disease, and infectious bursal disease.

Although the bursal disease is not fatal, the virus has some intriguing effects. For one thing, it selectively destroys lymphocytes and makes chickens resistant to infection by the lymphoid leukemia virus. Was this virus a friend or a foe? Would a vaccine based on this virus protect chickens from leukemia by destroying the colonic bursa, a lymphoid organ in the intestine where the leukemia virus multiplies?

Further work doused cold water on these hopes, even as it began to expand biomedical knowledge. The immunosuppressed chickens indeed proved less likely to contract lymphoid leukemia, but they showed signs of being highly susceptible to Newcastle disease and some bacterial diseases. The bursal disease was somehow giving other deadly pathogens a boost in overcoming the chickens' immune systems.

These studies were important in validating a just-emerging theory that T-cells and B-cells were functionally distinct arms of the immune system.

The methods for ultrastructural detection of virus in tissue adapted by Cheville in these studies have since become standard worldwide.

Later, Cheville began looking at the cellular pathogenesis of bacterial diseases and, his current assignment, brucellosis of cattle. Using specialized techniques to isolate different components of bacteria, his group has identified a unique intracellular life cycle for *Brucella abortus*, cause of undulant fever in humans.

Throughout his 26-year career with ARS, Cheville's many accomplishments have been documented by more than 170 scientific publications. As the merit of those publications has become known, his stature in the scientific community has grown.

He deserves special commendation for the emphasis he has placed on training other experimental pathologists. Through their accomplishments, the roads of inquiry that Cheville has opened have further broadened.

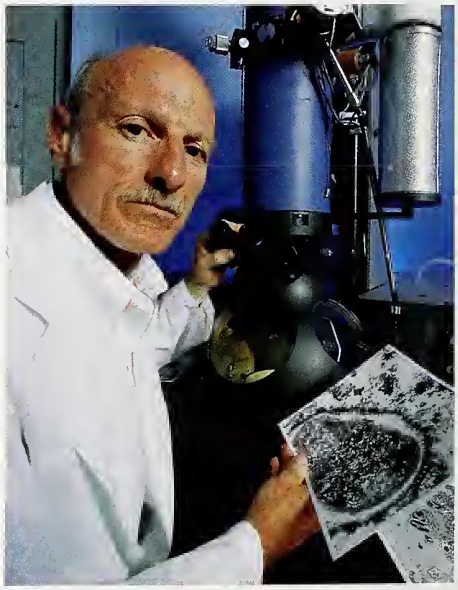
He has written several major textbooks, including *Introduction to Veterinary Pathology* and *Ultrastructural Pathology*, a treatise on electron microscopy.

On November 7, in Washington, D.C., Cheville received the Distinguished Scientist of the Year plaque and a $7,000 cash award. His research program at ARS' National Animal Disease Center in Ames, Iowa, will also receive an additional $40,000 in support funds.

This award is the most recent of many honors accorded Cheville throughout his notable career. A diplomate of the American College of Veterinary Pathologists in 1967 and a recipient of an honorary doctoral degree from the University of Liege in Belgium, Cheville is clearly preeminent among the world's animal health scientists.

The 1990 recipients of the agency's Outstanding Scientist of the Year awards are Wayne W. Hanna, a geneticist with the Forage and Turf unit in Tifton, Georgia; Thomas J. Henneberry, an entomologist at the Western Cotton Research Laboratory in Phoenix, Arizona; and G. Mark Holman, an entomologist with the Veterinary Toxicology and Entomology Research Laboratory in College Station, Texas.

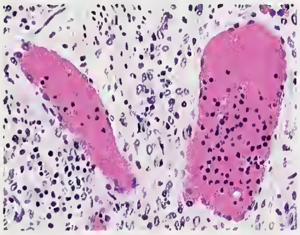
Regina Wiggen
Associate Editor



Norman F. Cheville

Agricultural Research

Cover: An Africanized bee swarm.
Photo courtesy of Scott Camazine, Cornell University.



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The Africanized Honey Bees

No longer must you go south of the border to get a peek at an Africanized honey bee.

NOAH PORITZ

Practically indistinguishable from their European cousins, Africanized bees (left) are more overtly defensive and more easily provoked than domestic honey bees (right). (K-3652-12)

On October 15, 1990, the first Africanized honey bee (AHB) swarm to migrate naturally to the United States was found at Hidalgo, Texas, near Brownsville. The swarm was destroyed, according to standard procedure, even before it was officially confirmed as Africanized. The official identification was made by Steve Sheppard, an entomologist with the Agricultural Research Service's Bee Research Laboratory in Beltsville, Maryland.

The swarm (a small one of about 3,000 bees) was found during a regular monthly check of the ARS traplines that are used to monitor the spread of Africanized honey bees.

Africanized honey bees are descendants of bees that were imported from Africa to Brazil in 1956. They were brought there by a Brazilian scientist who planned to cross them with the European-descended honey bees already present in South America to create a new strain of bees better suited to the Tropics. (Honey bees are not native to the New World and were originally brought here by European colonists.)

In 1957, the experimental insects were inadvertently released to the Brazilian countryside where they promptly interbred with resident honey bees, creating the offspring now called Africanized honey bees.

These hybrids have spread north and south from Brazil (see map); their current range extends from the middle of Argentina through Mexico and to the southern tip of Texas.

Neither African nor Africanized honey bees are the villains they've been made out to be under the name "killer bees." Although AHB's are more unpredictable and more overtly defensive than our domestic honey bees, they generally react only when their nests are threatened.

While AHB's tend to defend their colonies more vigorously, stinging in greater numbers and with less provocation, their venom is no more poisonous than that of the domestic honey bee.

"Although the current find is the first time Africanized honey bees have entered the United States by a natural expansion, it isn't the first time they've made an appearance in the country," says Al Dietz, an apiculturist with USDA's Animal and Plant Health Inspection Service (APHIS) based in Hyattsville, Maryland. APHIS is responsible for controlling foreign pest introductions in the United States.

Africanized honey bee swarms have hitchhiked on ships coming from Latin America into U.S. ports for many years. Since 1983, APHIS has destroyed 21 such swarms.

To monitor and slow the northward spread of AHB's, APHIS, with the Mexican government, established a cooperative program of swarm trapping, queen marking and replacement, increased drone rearing, and public education. Since 1987, the cooperative program has placed more than 80,000 swarm traps in Mexico, capturing more than 25,000 Africanized honey bee swarms.

PERRY A. RECH

Entomologist Steve Sheppard scans magnified images of bee wings into a computer that will calculate the probability that wings were from an Africanized bee. (K-3735-8)

A similar effort was launched in the Rio Grande Valley of Texas in the summer of 1990. There, the APHIS trap effort consists of monitoring 285 bait hives, covering hundreds of miles.

APHIS, ARS, and Texas A&M have established traplines in Texas to observe changes in the resident population of honey bees as they spread. APHIS is also committed to supporting Texas in eliminating the initial AHB swarms found in the state.

"Only a few swarms are expected to enter the country this year," says Ralph Bram, ARS national program leader for medical and veterinary entomology research. "It will be a year or more before many swarms come across."

How far north into the United States the bees will eventually spread is not yet clear; many different predictions exist, according to Bram.

The effect of the United States' extensive bee management, combined with the cooler climate in much of North America, makes it difficult to predict future population sizes. Some experts believe that the Africanized bees won't be able to take cold winters, which will limit their spread north. Others believe the Africanized intruders will be able to survive anywhere that honey bees now live in North America.

A Setback for Pollination and Honey

U.S. agriculture and beekeeping industries have two primary concerns about the intrusion of the AHB into this country. Domestic honey bees that interbreed with them may become harder to manage as pollinators of crops and may not be as efficient when it comes to honey production, according to Bram.

About one-third of the American diet is directly or indirectly dependent on crops that are pollinated by honey bees.

"Honey bees are often trucked from place to place to ensure the pollination of all types of crops—crops worth $10 billion a year, including apples, berries, cantaloupes, cucumbers, and almonds. They also

produce about $150 million worth of honey a year," Bram says.

ARS has 13 scientists at 4 locations conducting AHB-related research. They are also involved in cooperative projects with APHIS, the Cooperative State Research Service, and the Extension Service, as well as state, local, and industry officials.

Among the major tasks of the research is how to identify an Africanized honey bee and how best to manage honey bees in areas where AHB's intrude.

Identification is one of the basic responsibilities of the ARS Bee Research Laboratory in Beltsville, Maryland. As the lab that officially labels suspected bees as Africanized or not, it provides identification services 24 hours a day, 7 days a week to local, state, and federal officials.

"AHB's cannot be distinguished from their European cousins by the untrained eye, although AHB's are slightly smaller," says microbiologist Hachiro Shimanuki, who supervises the laboratory. "It requires sophisticated training to tell the two types of bees apart."

ARS relies primarily on a two-stage procedure to identify the bees. The first method, called Fast Africanized Bee Identification System (FABIS), depends on wing measurements. The method was developed by insect geneticist Thomas Rinderer and his colleagues at the Honey Bee Breeding, Genetics, and Physiology Laboratory in Baton Rouge, Louisiana. It is based on the fact that the wings of European honey bees are minutely larger than those of AHB's.

Samples not eliminated as European with FABIS undergo computerized morphometric analysis, which measures many parts of the honey bee, exploiting tiny differences.

Shimanuki and other researchers are also working on identification techniques based on DNA analysis and chemical and immunological differences.

JACK DYKINGA

Using the Fast Africanized Bee Identification System, geneticist Thomas Rinderer checks the forewings of bees from a suspect colony. (K-3771-6)

Entomologist Akey C. Hung has recently isolated two proteins that are distinct to the Africanized honey bee. Using these, he expects to develop an identification test kit that can be used in the field.

"Now that the proteins have been isolated, the technology already exists to create the kit," Hung says.

Genetic Mixing

An important question is what happens when Africanized honey bees mix with European-derived honey bees.

Considerable controversy exists on how much genetic mixing has actually taken place between African honey bees and resident honey bees. Originally, it had been hoped there would be a lot of interbreeding in South America and that this would dilute some of the African honey bees' traits, particularly their defensiveness.

But some experts claim that there has been little if any genetic interaction and that the honey bees now crossing the border are about as pure African, genetically speaking, as the original bees that were loosed in Brazil 33 years ago.

Steve Sheppard, the ARS entomologist charged with making official identifications of suspected Africanized honey bees, recently made several trips to South America to the geographical transition zones where Africanized and European honey bee territories overlap.

"This is where there should be the greatest amount of genetic mixing ranging from European to hybrid to African, if such mixing is taking place," Sheppard says.

Using samples from the transition region, he has been comparing inherited markers from mitochondrial DNA, which comes solely from the queen, and from nuclear DNA, which is inherited from both parents.

"There is no question that I found honey bees that were hybrids—ones with genes from both African and European honey bees," Sheppard says.

Sheppard and his coworkers also found bees that ran the gamut of behavior from gentle to very aggressive within the transition zone.

"When we compared identification of a honey bee as European or Africanized with its behavior, we found bees that were Africanized by definition but were as gentle as any European honey bees as well as honey bees that were European by definition but very aggressive," Sheppard says.

ARS scientists expect it will be possible to mitigate the Africanized honey bees' undesirable traits by arranging for more European honey bees in the breeding mix.

By controlling about 95 percent of the matings of queens, it should be possible to prevent AHB's from dominating an area, said Rinderer and entomologists Jon Williams and Richard Hellmich, who are also at the ARS lab in Baton Rouge.

During several pilot tests, Hellmich found that flooding an area with drones from controlled hives during mating season could control the mating choice of free-flying queens 95 to 99 percent of the time.

If beekeepers can control matings at least 90 percent of the time by drone flooding and making sure that an Africanized queen has not taken over a managed hive, "then you're okay" in terms of maintaining hive manageability, Hellmich says.

Last spring, the ARS scientists began planning a cooperative project with officials in Guatemala to test the drone-flooding technique in areas where AHB's have already spread.

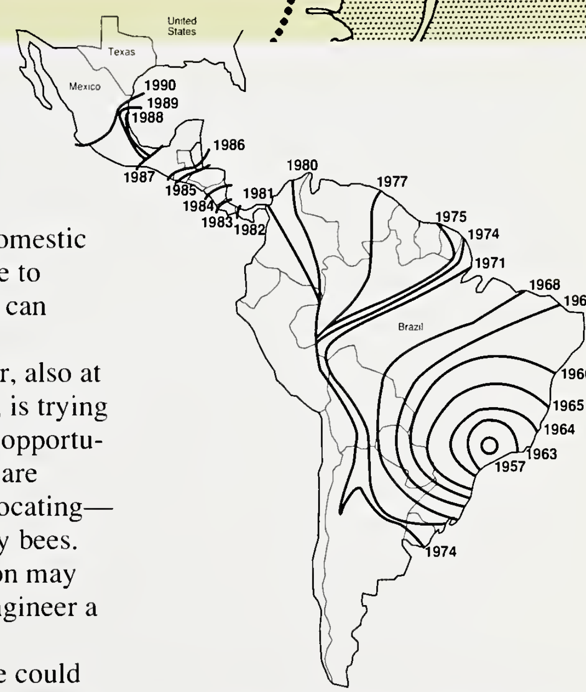
"Hopefully, in the coming year we will be able to establish experimental breeding apiaries in the highlands and the lowlands to test the drone-flooding techniques," Rinderer says.

Building a Better Bee

Another way to protect domestic honey bee colonies would be to develop a strain of bees that can outcompete AHB's.

Geneticist Allen Sylvester, also at the Baton Rouge laboratory, is trying to set the stage for just that opportunity. He and his colleagues are mapping—identifying and locating—the genes of European honey bees. In the future, this information may allow them to genetically engineer a superior bee.

"The ideal would be if we could either develop a honey bee that could directly outcompete or outbreed an Africanized honey bee or create a honey bee that is resistant to a dis-



A web of traplines stretches through Texas to the north of Hidalgo where the first Africanized honey bees in the U.S. were captured. Accidently released in Brazil, the hybrid bees have expanded northward and southward since 1957.

JACK DYKINGA

Biological lab technician John Edwards at the Carl Hayden Bee Research Center in Tucson, Arizona, vacuums in a sample of honey bees from a wild colony in a dead saguaro cactus. (K-3776-18)

PERRY A RECH

Microbiologist Hachiro Shimanuki is in charge of the honey bee research lab in Beltsville, Maryland, which is responsible for the official identification of Africanized bees. (K-3736-15)

ease that the AHB's would succumb to," Sylvester says.

Baiting Bad Bees

A genetically engineered honey bee is only a possibility for the distant future. On the more immediate horizon at the Baton Rouge lab is the search for ecologically sensitive chemical baits that can be used right now to control the presence of undesirable bees, especially in public places, without doing in other bees.

"We're looking at both natural and synthetic chemicals that will kill or repel honey bees without leaving residual compounds that might harm the environment," says Rinderer.

Researchers at the Baton Rouge lab have devised one system that fits the bill. A sugar syrup bait is placed in the area, and the honey bees that are drawn to it are checked for

Africanized traits. If Africanized honey bees have responded, a small amount of toxin could be added to the bait for an hour or two to kill the visiting bees.

"A system like this, with no general spraying involved, would cause minimum risk to the environment," Rinderer says.

Insect geneticist Anita Collins has also been working on repellants, first at the Baton Rouge lab and now at the Honey Bee Research unit in Weslaco, Texas.

DEET, developed by ARS in the 1950's as a mosquito repellant, was also found to repel honey bees. Honey bees recovered from exposure eventually, but DEET did cause them to stop stinging completely, a pause that could buy time for people to move away.

But to be most effective, DEET has to be sprayed in the air at the time of attack. In tests, DEET sprayed on the skin or clothes did not deter aroused bees.

More techniques that can be used to control AHB colonies without

JACK DYKINGA

Entomologist Gordon Waller examines a marked drone that will be recovered to determine distances traveled in mating flights. (K- 3775-2)

Stingometer Finds Savage Stingers

A honey bee temper tester, or stingometer, may help protect us from Africanized honey bees.

"By spotting bees that have Type A personalities, we will have a better chance to control the more aggressive strain of honey bee that just moved into Texas from Mexico," says Hayward G. Spangler with the Agricultural Research Service.

Spangler invented an electronic device to record how many times disturbed bees hit a target while attempting to sting it, an indication of their demeanor. Africanized honey bees often sting many times. European honey bees, the strain in this country, usually inflict only one or a few stings when provoked.

The device consists of a small plastic bottle (target) with a sensor inside. A cable connects it to an electronic device to record how many times disturbed bees hit the target while trying to sting it. Hung in front of a hive entrance, it records for 10 seconds after the bees have been disturbed.

An early prototype was used in Costa Rica to test with Africanized honey bees. The device recorded up to 24 hits per second. That compares to about four hits per second for domestic bees near Spangler's lab in Tucson, Arizona.

Once bee officials locate a hostile hive, they can either destroy it or replace the mean old queen with a gentle one who will produce friendlier offspring.

JACK DYKINGA

A honey bee temper tester is put through its paces by entomologists Hayward Spangler (foreground) and Eric Erickson. The number of stings electronically recorded on the black bottle helps determine how defensive the colony is. (K-3773-14)

Spangler and fellow ARS entomologist Eric H. Erickson have applied for a patent and are hoping private industry will develop the device into a commercial product. Spangler hopes the commercial version will cost no more than $250 and will prove useful in dealing with highly defensive Africanized honey bees.—By **Dennis Senft,** ARS.

Hayward G. Spangler and Eric H. Erickson are located at the USDA-ARS Honey Bee Research Laboratory, 2000 East Allen Road, Tucson, AZ 85719 (602) 670-6380.

harming beneficial insects are being worked on at the Weslaco lab.

"In preliminary experiments, we found that a spray of water mixed with dishwashing detergent did not cause a flareup by the bees and killed them several minutes after they were sprayed," says Collins. "But spraying honey bees with streams of diesel fuel, plain pressurized water, or a carbon dioxide fire extinguisher did not kill them."

ETOC, which is an experimental, synthetic pyrethroid—an insecticidal chemical—has also shown promise for controlling swarms.

"The main thing really though is not to bother bees of any sort," says Collins. "If you find bees nesting near people or penned animals, you need to get assistance from trained professionals to remove colonies before they become pests. Call your fire department or extension agent for help."

Mark Your Queen

Another technique that will help beekeepers maintain control of the type of honey bees in their hives is queen marking.

Africanized honey bee queens will often take the place of the resident European queen. They breed faster, and the offspring reach maturity sooner than European honey bees. And of course, the offspring may carry the traits of increased defensiveness and less efficient pollination and honey production.

Beekeepers are being advised to mark their queens with a small dot of paint or nail polish so that they can be sure that a desirable queen is in residence.

"If an Africanized queen has taken over the colony, it should be replaced with a queen known to be of European stock," Collins says.

JACK DYKINGA

JACK DYKINGA

Geneticist Anita Collins uses an insect repellent containing DEET as a direct spray to disperse attacking bees. (K-3760-12) and (K-3760-13)

Building A Better Bee Trap

Out West at the Carl Hayden Bee Research Center in Tucson, Arizona, ARS scientists have been busy developing traps to attract and eliminate Africanized honey bees. They are also looking for methods to measure the bees' hostility and treatments for animals and people that might be stung with enough venom to have toxic reactions.

Traps made of durable wood pulp are charged with pheromone to lure swarms of bees. These traps have already been proven effective in Arizona, California, and Texas and in Costa Rica with Africanized honey bees.

"These traps can be a useful means of mass-trapping unwanted Africanized honey bee swarms out of an area and, if the traps are properly deployed, they can be a powerful means of control," says entomologist Justin Schmidt.

Hayward Spangler is working on a device to measure the hostility of honey bees to help determine whether a colony is gentle or hostile, and whether it should be maintained or eliminated because of its behavior. *[See* Stingometer, *page 9.]*

His intention is to have a battery-operated, handheld device that can be carried into apiaries or the field to

JACK DYKINGA

Near Ciudad Victoria, Mexico, ARS chemist Raul Rivera (foreground) and Mexico Department of Agriculture technician Jose Zavala collect bee scout traps. Similar traps have been set up in southern Texas along the Africanized bees' predicted entry route into the United States. (K-3752-13)

give beekeepers and inspectors an almost instant answer.

As part of the quest for information about how honey bees may colonize an area, scientist Gerald Loper has been tracking drone bees with radar as the drones search for mates. He hopes to learn exactly how far and where a queen goes to find a group of males.

Researchers at the lab are also trying to build a census of honey bees in Texas. Entomologist Eric Erickson has been mapping the distribution of wild colonies in the state and investigating their characteristics and habits.

"This kind of information will help beekeepers anticipate how the Africanized bees may spread in their area," says Erickson. "In order to know what changes take place in the local population after the Africanized honey bees arrive, we need to know what is going on now—how many honey bee colonies exist, where they are, and what they are doing."

Fear Not the Swarm

Africanized honey bees are not a marauding danger to the general public, although they are more easily provoked to stinging than are European honey bees. They will sting in greater numbers and are likely to follow an attacker farther. But swarms are not out searching for victims to sting.

"It's not so much that they're more aggressive as that they're more defensive," says Collins.

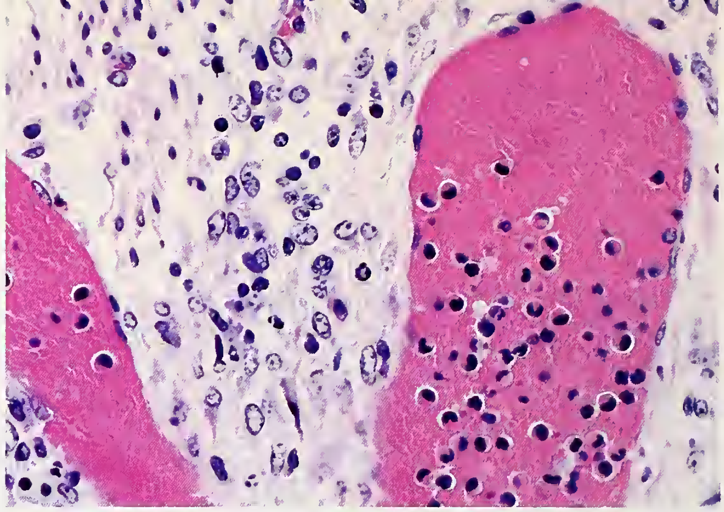
People will most often see swarms of honey bees, which are groups of bees out looking for new nest sites. Swarms are not likely to sting because they have no nest to defend. This goes for Africanized as well as European honey bees.

Chances are, a person who doesn't see honey bee swarms or colonies nowadays won't be more likely to since the AHB's have arrived.

There will probably be more stinging incidents once Africanized honey bees become fully established in the United States, but the chances of being fatally stung by any stinging insect, AHB's included, remain less than the chances of being killed by lightning.—By **J. Kim Kaplan,** ARS.

Ralph Bram, USDA-ARS National Research Program Leader for Entomology, is in Room 211, Bldg. 005, BARC-West, Beltsville MD 20705. Hachiro Shimanuki, Akey Hung, and Steve Sheppard are at the USDA-ARS Bee Research Laboratory, Beltsville, MD 20705 (301) 344-3970; Tom Rinderer, Jon Williams, Richard Hellmich, and Allen Sylvester are at the USDA-ARS Honey Bee Breeding, Genetics, and Physiology Research Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820 (504) 766-6064; Anita Collins is at the USDA-ARS Honey Bee Laboratory, P.O. Box 267, Weslaco, TX 78596 (512) 969-2511; Eric Erickson, Gerald Loper, Justin Schmidt, and Hayward Spangler are at the USDA-ARS Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ 85719 (602) 670-6709. ◆

Rare Disease Shared by Humans and Cattle



High magnification of blood vessels of the intestinal lamima propria illustrate a hallmark feature of LAD patients — extremely high numbers of neutrophils (black spots inside the pink areas) inside the blood stream and rare extravascular neutrophils in infected tissue. (K-3875-1)

Human leukocyte adhesion deficiency (LAD) was first diagnosed by medical researchers in 1982. Since then, about 100 cases have been reported. It is almost always fatal unless the victim can receive a bone marrow transplant.

White blood cells in people or animals with this disorder lack a protein known as MAC-1, which is needed to penetrate blood vessel walls. Without this protein, neutrophils, one of several white blood cell types, are unable to leave the bloodstream and reach infection sites in other tissue. Thus, the individual's immune system is weakened and the body is left defenseless against invading bacteria.

In an infant with this disorder, virtually any infection—even one from an ordinary event like cutting teeth—could prove fatal.

While researchers on human LAD strongly suspect the disease could be cured by inserting normal genes into the white blood cells in bone marrow, the lack of animal models has hampered this work.

Now, Marcus E. Kehrli, Jr., of USDA's Agricultural Research Service, believes that the disease can be studied in cattle.

Kehrli, who is a veterinary medical officer at the agency's National Animal Disease Center in Ames, Iowa, says, "The condition may be more common in dairy cattle than in humans."

It was Kehrli who recognized the similarities between a Holstein heifer calf that died at the center in 1989 and the symptoms of human LAD.

He traced the heifer's ancestry back for six generations, finding that her pedigree traced three times to one of the most prominent sires of the Holstein breed.

Further studies of pedigrees of other calves reported to have a previously described granulocytopathy syndrome have demonstrated that all calves are related to each other and that they all trace back to that one Holstein bull.

Kehrli reported his findings in the November issue of the American Journal of Veterinary Research.

His work so far provides the basis for developing a laboratory diagnostic test that will identify carrier animals. "We hope to have the diagnostic test ready in about 2 years," says Kehrli. "But how soon a test is ready depends on how soon we can reproduce this trait in another calf so we can isolate and sequence the defective gene."

To get another MAC-1-deficient calf, he and coworkers are superovulating eggs from the Holstein calf's mother who is a carrier of the trait. Superovulation provides as many as 10 to 15 eggs at a time that can be fertilized and implanted in other cows for gestation. The odds are high that the fatal condition will turn up in one out of four of the embryo transfer calves.

While researchers wait for the surrogate mothers to give birth, they're sequencing the DNA of the normal gene so they can compare it to the defective gene. They will then attempt to copy the normal gene and place it into a harmless virus, which in turn is used to infect bone marrow cells from a MAC-1- deficient calf. These bone marrow cells, now carrying a copy of the normal gene, will be once again placed into the affected calf.

"We hope the cells carrying the normal gene will establish themselves in the bone marrow, thus producing the much-needed MAC-1 protein," says Kehrli. This technique has been successful in human tissue culture studies done by medical researchers in Houston, Texas, Ann Arbor, Michigan, and Boston, Massachusetts. But the lack of an animal model prevented putting the cells into living bone marrow.

"If Kehrli succeeds in fixing the genetic defect in cows, medical researchers may consider using similar therapies with humans," says pediatrician Donald C. Anderson at Texas Children's Hospital in Houston. In collaborative studies with Kehrli, Anderson has screened the calf's relatives' blood for the MAC-1 protein on white blood cells.

"New therapies are needed to provide alternatives to bone marrow transplants, which are always risky for the patient," says Anderson.—By **Linda Cooke,** ARS.

Marcus E. Kehrli, Jr., is at the USDA-ARS Metabolic Diseases and Immunology Research Unit, National Animal Disease Center, P.O. Box 70, Ames, IA 50010 (515) 239-8462. ◆

The Cranberry Girdler: Sex Is Its Undoing

By tricking an inconspicuous, beige moth with a mating lure, ARS scientists have helped farmers and nursery growers who raise two crops popular in December—cranberries and Christmas trees.

The lure was first developed, however, to help grass seed growers in the Pacific Northwest. Over a decade ago, ARS entomologist James A. Kamm and chemist Leslie M. McDonough began working on the sex pheromone of cranberry girdler *(Chrysoteuchia topiaria)* to detect the moths, whose immature, or larval, stage inflicts damage on the crop.

Growers use the pheromone traps to determine whether they'll need to use insecticides. The girdlers sporadically invade fields of both forage and turf ryegrasses.

Today, the trap is used in cranberry bogs, grass fields, and tree nurseries to track infestations by monitoring the adult girdlers.

The wormlike girdlers cut cranberry yields by damaging the plant's root system. In the Pacific Northwest, it also attacks Douglas-fir seedlings intended for reforestation programs and Christmas tree farms.

Adult girdlers emerge from silk-lined underground cocoons in June. Male moths locate mates by tracking the sex pheromone that the females emit from an abdominal gland.

After mating, the female lays her eggs in the plant canopy. A week or two later, the eggs hatch into larvae that chomp around the outer layer of underground roots, leaving a girdle of damaged tissue that in effect starves the plant.

Girdlers don't hit every field every year, says Kamm, who works at the National Forage Seed Production Research Center in Corvallis, Oregon. "The advantage of the pheromone trap is that it detects infestations when they do occur, so growers can apply insecticides."

USDA Photo

Today, the trap is used in cranberry bogs, grass fields, and tree nurseries to track infestations by monitoring the adult girdler.

The trap consists of a small red rubber plug impregnated with a synthetic version of the female's pheromone. The plug is suspended over a sticky trap. Attracted males try to mate with the plug and become trapped when they fall in the "stickum" in the trap.

Several companies manufacture and sell the girdler pheromone trap throughout the United States. A significant proportion of the sales are in Massachusetts, New Jersey, Maine, Oregon, Washington, and Wisconsin, where the crops attacked by the girdler are grown.

Around Grayland, on the southern Washington coast, about 40 percent of the cranberry growers use girdler pheromones as part of an integrated pest management program aimed at cutting pesticide use, says Kim Patten, who manages the Washington State University Cranberry Research unit in Grayland.

Besides advising county extension agents in Oregon and Washington on how to use the traps in cranberry bogs, Kamm collaborated with USDA's Forest Service, the Oregon Department of Forestry, and Weyerhaeuser Corporation for implementing their use in tree nurseries.

Certain grasses are resistant to the girdlers, however, and grasses infected with an endophyte (a plant living within another plant, in this case a fungus) are also not attacked by the girdler. Planting these resistant or nonhost grasses near cranberry bogs and tree nurseries could cut down moth populations, says Kamm.

Such measures to minimize pesticide use will protect species that benefit the crops. Bees, for instance, that pollinate cranberries can be harmed by pesticides, as can swallows, one of the girdler's natural enemies.—By **Julie Corliss,** ARS.

James A. Kamm is at the USDA-ARS Forage Seed and Cereal Research Unit, 3450 Northwest Orchard Ave., Corvallis, OR 97331-7102 (503) 757-4365. Leslie M. McDonough is at the USDA-ARS Fruit and Vegetable Insect Research Unit, 3706 Nob Hill Blvd., Yakima, WA 98902 (509) 575-5970. ◆

Modern Air May Favor Rangeland Brush

Ecologist Wayne Polley uses a surface neutron meter to monitor moisture levels in various parts of t

On the blackland prairie of central Texas, midway between Houston and Abilene, the Old West hangs heavy in the air.

In fact, one spot on the prairie boasts what might as well *be* the air of the Old West—specifically, air with a carbon dioxide (CO_2) content of about 265 parts per million.

It's not that time stands still on the Texas prairie. This relatively pristine air—today's CO_2 levels edge closer to 350 ppm—is carefully cultivated in a sort of "time tunnel" maintained at Temple, Texas, by Herman Mayeux, Hyrum Johnson, and Wayne Polley.

They're not nostalgia nuts; they're U.S. Department of Agriculture scientists. They believe it is possible to see into the future—and see what a potential increase in CO_2 levels may do to plants—in part by studying the past.

Working at the Grassland, Soil, and Water Research Laboratory operated at Temple by USDA's Agricultural Research Service, the three scientists in 1988 constructed a serpentine growth chamber 128 feet long and about 5 feet tall.

Underneath a tight canopy of clear plastic, air flowing through the growth chamber gradually declines in CO_2 content from today's 350 parts per million to as little as 150.

By growing assorted plants at different CO_2 levels, the scientists can observe the effects of changing CO_2 on plant productivity and water-use efficiency, including the ways atmospheric change over the years may have altered the vegetative face of America.

Simply determining what kind of air early Americans breathed has been an international affair. Since the 1950's, atmospheric carbon dioxide levels have been monitored regularly at Hawaii's Mauna Loa volcano. But for information on earlier days, Johnson turned to the findings of Swiss, French, and Russian researchers who studied 7,000-foot-long ice cores from Greenland and Antarctica.

As ice freezes, it tends to trap air. Air can be extracted from the ice cores and the carbon dioxide content measured. Using these techniques, the researchers have been able to calculate air quality as far back as 160,000 years ago. Comparison of more recent layers with information from measurements in Hawaii has shown the answers are compatible, Johnson says.

Perhaps ironically, for their own experiments the ARS scientists have had to draw on the latest in 20th-century high tech to mimic 19th-century nature.

As air with today's CO_2 concentration enters the growth chamber at one end, the CO_2 is used by plants in photosynthesis as it passes along so that the air has progressively less carbon dioxide.

The rate at which plants use carbon dioxide in photosynthesis depends on the intensity of sunlight. Since light is such a crucial factor, sensors on the roof of the greenhouse surrounding the growth chamber indicate if a cloud is passing over.

If the light changes, fans are signaled to slow their pushing of CO_2-laden air through the passageway, giving the plants at the "modern" end more time to take in the CO_2. Computers monitor and control the airflow and monitor other variables in the growth chamber.

Air temperature and humidity are also crucial, so cold-water coils and heating elements are stationed every 25 feet along the passageway to maintain the correct levels.

owth tunnel. (K-3833-16)

PERRY A. RECH

"Plant stomata—openings in the plant's leaves—are quite sensitive to the amount of humidity," Johnson says. "If the air is dry, these stomata close, and that affects the rate at which the plants take up CO_2."

Is the information to be gleaned really worth all this trouble? If you're in the market for some good news, the answer is "yes," says Johnson.

"During the period from around 1850 to the present, CO_2 levels in the atmosphere increased about 30 percent," he notes. "In one of our first experiments using the tunnel, we grew wild mustard and oats at a range of CO_2 levels from below those of the mid-1800's to today.

"We saw a tremendous response to higher CO_2 in both species. When we compared plants' biomass yields when grown in the tunnel at the CO_2 levels of 1860 to yields for plants grown with today's levels, the increase in yields again was about 30 to 50 percent. So for each 1-percent increase in CO_2, we've seen at least a 1-percent increase in biomass."

PERRY A. RECH

Ecologist Hyrum Johnson observes vegetation inside a "time tunnel". (K-3831-9)

Stated plainly, plants flourish in higher CO_2; it has a fertilizing effect on them. However, some flourish more than others, and that could change the look of the range, according to Johnson.

"Most plants are what we call C3 types," he explains. "This includes oats, wild mustard, and most crops, but also woody brush plants like mesquite. Then there are the C4 plants, the warm-season grasses like sorghum, corn, and sugarcane, as well as the majority of our native forage grasses.

C3 and C4 plants are classified as such because of the chemical makeup of acids they produce during photosynthesis. The first acid produced by a C4 plant contains four carbon atoms; the initial acid from a C3 plant has three. While C4 plants may produce a three-carbon acid later in the photosynthetic process, and C3's are capable of producing four-carbon acid, they derive their names from the initial product.

"The C3's increase photosynthesis more under higher CO_2 levels than C4's do. There's a close relationship between the amount of photosynthesis taking place and the amount of plant growth. So C3 plants benefit more from the additional CO_2, allowing them to dominate the vegetation."

The same upward spiral of CO_2 that began some 150 years ago, primarily because of burning fossil fuels and felling forests, is expected to push atmospheric carbon dioxide to twice its current level in the next century, Johnson says.

But the relative advantage this could give to C3 plants doesn't mean the American West is doomed to become one giant mesquite patch, says Johnson.

"Both C3 and C4 plants respond to CO_2 in a positive way by improving water-use efficiency—the amount of plant productivity per amount of water used," he says. "But C4 plants are always more water-efficient."

This improved water-use efficiency could open the door to production of certain desirable plants in areas that would previously have been deemed too parched. Irrigation needs should also decline in the presence of high CO_2, according to Herman Mayeux.

"When plants open their stomata to let CO_2 in, that also lets water escape from the plant," says Mayeux.

"If CO_2 levels are low, the stomata must open wide and the plant can lose a lot of water. But when there's lots of CO_2 available, they don't have to open as much."

This was demonstrated in the 1989 study with oats and wild mustard, Johnson notes.

"At one end of the tunnel, where CO_2 levels were only 150 ppm, the plants were using water extravagantly," he recalls. "The plants at the other end of the tunnel, where CO_2 was 350 ppm, were losing only about half as much water.

"If CO_2 levels do rise as predicted, I think we'll see even greater amounts of plant production on the range, perhaps of both types—C4 as well as C3. The greatest benefit of higher CO_2 for a C4 plant on rangeland is improved water efficiency, so the plant could grow longer.

"Still, there are going to be places where the C3's get such a big boost by benefiting more from the higher CO_2 that they'll simply outcompete the C4's," Johnson adds. "We think that's what is happening now on the range.

"But the increased water-use efficiency of C4 perennial grasses may mean they'll be more productive if reseeded on the rangelands after clearing the brush. Also, C3 range grasses dominate some rangelands, and these important plants have almost certainly benefited from increasing CO_2."

Earth's atmosphere is increasing in carbon dioxide content, and scientists are looking at the consequences.

Certainly, the changes on the range so far haven't been cause for celebration among ranchers. Direct agricultural losses attributed to brush in the American West are calculated to total some $1.7 billion each year, without taking into account livestock losses to plant poisonings.

PERRY A. RECH

Range scientist Herman Mayeux observes light-sensing bars that indicate solar radiation levels within the growth tunnel. (K-3829-12)

In the last 40 to 50 years, millions of acres of rangeland have been cleared, root-plowed, or sprayed with herbicides, but it has appeared to be a losing battle.

According to surveys by USDA's Soil Conservation Service, Texas had 88 million acres of brush-infested range in 1963. Ten years later, despite the wide array of control measures used, the total was up to 92 million acres. By 1982, the brush had spread across 105 million acres.

Woody species have always had their place on the range. But in presettlement times, they often appeared in widely separated sites or as individual plants sprinkled sparsely across otherwise pure grasslands.

A classic case in point is the Jornada Experimental Range in New Mexico. When surveyed and described by the U.S. Land Office in 1858, no shrubs were evident on 60 percent of the range's approximately 143,318 acres. Only 5 percent of the area contained dense mesquite, and there were no dense stands of creosotebush or tarbush.

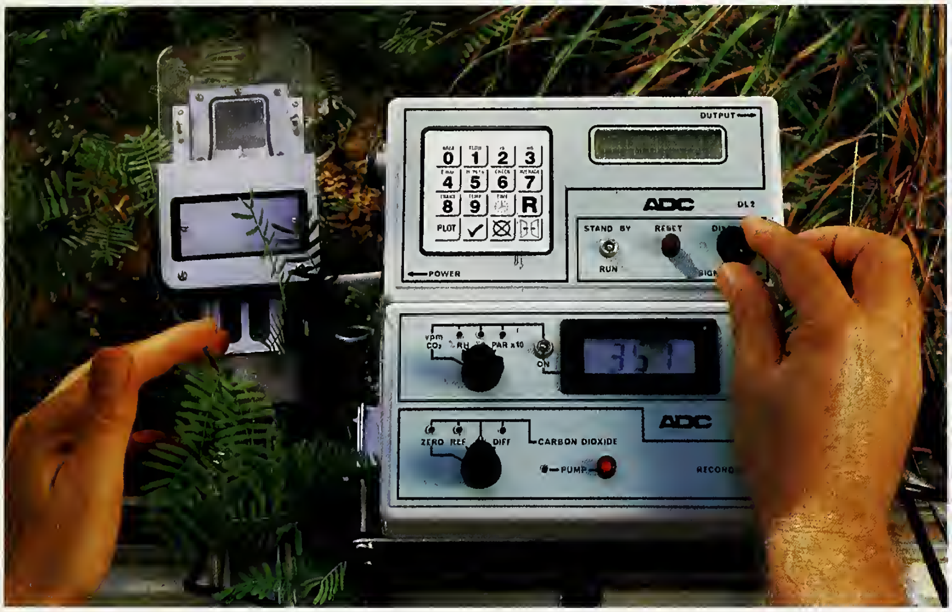
But by 1963, none of the area was free of woody species; 73 percent was dominated by dense stands of mesquite, creosotebush, or tarbush.

This change from open C4 grasslands to C3 shrublands has been swift in a historical sense, occurring since CO_2 levels began to rise.

The North American grasslands are believed to have been in place for thousands of years, but it took less than 150 years for the woody plants such as mesquite and creosotebush to win out. This has also occurred on former C4 grasslands of other continents such as South America, Africa, and Australia, suggesting that some global change such as increasing atmospheric CO_2 is involved.

Nor will farmers and ranchers be the only ones affected by the change in plant cover. Unwanted woody

PERRY A. RECH



Rate of photosynthesis and transpiration is measured by a portable carbon dioxide analyzer. (K-3828-13)

plants on watersheds may use precious water that might otherwise go for irrigation, industry, or urban use—a loss that could limit population growth in the West.

"I think in general, without some help, brush will continue to increase on rangeland," Johnson says.

"But if CO_2 levels go up, because of the plants' greater capacity for growth, it's possible this rangeland could be managed in some more intensive way.

"With the possibility of increased capacity for growth and increased water-use efficiency, it might be feasible to more carefully manage the land for greater productivity. The problem now is to determine how we can take advantage of the improved productivity of rangeland vegetation."

Despite boosts in plant growth and water-use efficiency, higher CO_2 levels do not represent an endless vegetative bonanza. Mayeux and Johnson say plants' positive response to the extra carbon dioxide tends to hit a plateau.

"You see a much greater response at lower levels of increasing CO_2, such as happened in the last century," says Johnson. "C4's tend to level off at 350-400 ppm, but C3's can continue to respond at 1,000 ppm and beyond.

"Plants level off because of their biochemical limits; you can literally swamp their photosynthetic capability. There are limits to how far a plant can chemically and physically change in response to higher CO_2, and the limits aren't the same for every species."

"We might want to look at adjusting our livestock operations to use more brush," he says. "Some animals can thrive on it. Goats graze mesquite, and probably so will several other meat-producing species." Ranchers in the Southwest are already expressing interest in raising more goats, he adds.

Meanwhile, the researchers will continue to use their growth chamber as a sort of crystal ball to determine how different plants will react to a new world. Currently, they're comparing the performances of honey mesquite, a C3 plant, and little bluestem, a C4 that's one of the main prairie grasses.

"I'm preparing for a surprise on mesquite," Johnson says. "I think it might act differently from other C3's. So we need to try to classify plants beyond just C3 or C4, into groups that exhibit the same kind of response to changing CO_2."

Neither Johnson nor Mayeux looks to the future with any great apprehension. In their eyes, the opportunity outshines the danger.

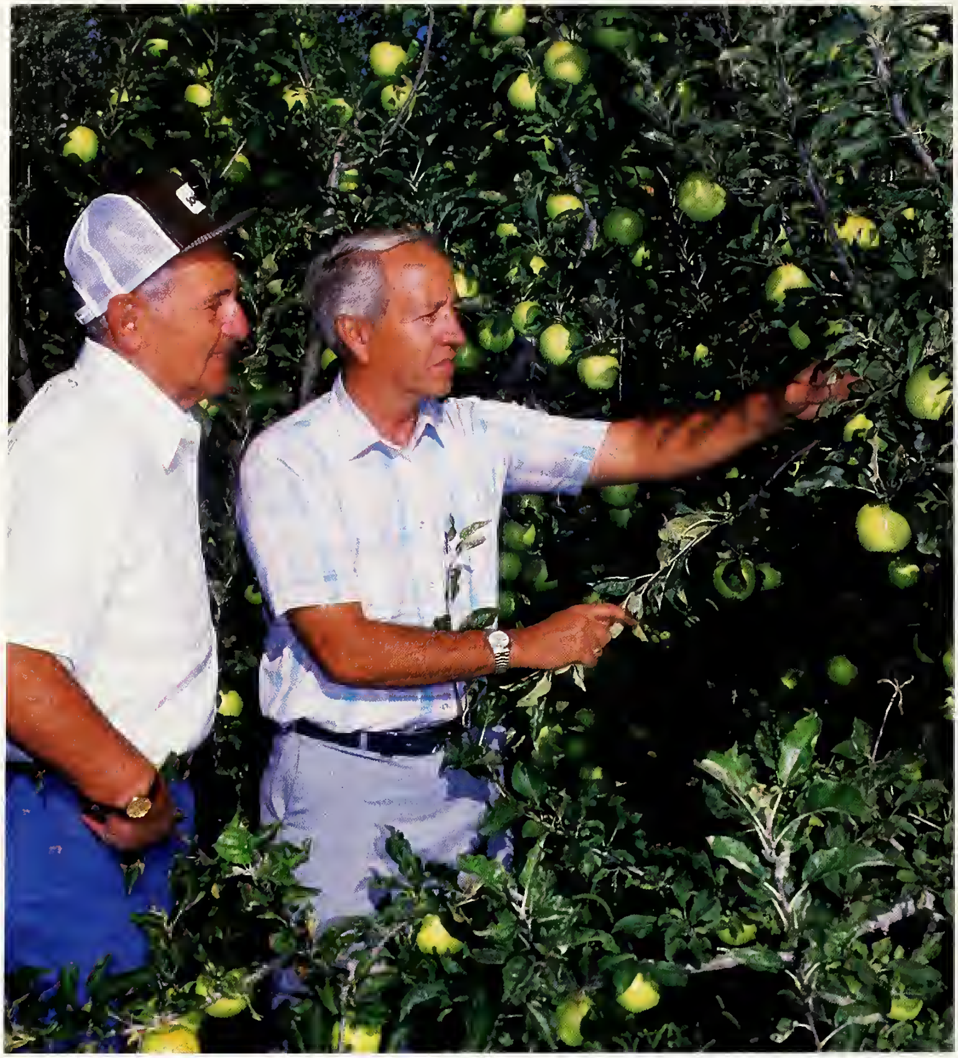
"I don't anticipate any great catastrophe," Johnson says. "In fact, the overall effect may be highly beneficial. I think the main thing is to understand plants' responses to increasing CO_2, the environmental relationships, and where the plants fit in. If we understand this system, we can make it work to our benefit.

"I think there will be a place in the foreseeable future for C4 plants on the range. But it may not be where we're used to seeing them grow."

"How the range will look in 100 years will depend in large part on how successful we are at maintaining grass and whether we persist in the traditional uses of the rangeland to produce food and fiber," Mayeux adds. "Given what we think will happen, the traditional uses in terms of grazing will get harder. But there are other options."—By **Sandy Miller Hays**, ARS.

Herman S. Mayeux Jr., Hyrum B. Johnson, and H. Wayne Polley are in Grassland Protection Research at the USDA-ARS Grassland, Soil, and Water Research Laboratory, 808 E. Blackland Road, Temple, TX 76502 (817) 770-6500. ◆

DOUG WILSON



In an apple orchard in Quincy, Washington, plant physiologist Max Williams (left) and grower Joe Bullery examine chemically thinned Golden Delicious apples for fruit size and overall quality. (K-3849-1)

Biting Into Apple Research

At the core of the matter are better fruits, improved means of production, and updated ways to keep it fresh

Crunch into a shiny, red apple and savor its fresh taste and juiciness. Unlike many fruits, these fiber-rich snacks are readily available year round. Maybe that's why they're one of America's favorite fruits.

Americans ate more apples than ever before last year—about 20 pounds per person. A bumper crop in the fall of 1989 helped establish this record.

Nature may have set the stage for the performance, but careful planning and hard work by growers contributed a great deal to last year's record production of top-quality apples.

So did advice from ARS scientists, who for years have sought ways to keep the crunch in and the bugs out. They also investigate how to keep apples brightly colored and flavorful in storage and new ways to fend off molds and rots.

Picture, if you will, the well-tended fruit orchards of east-central Washington, where nearly half of the country's apples grow. The region's long growing season with warm days and cool nights, its fertile soils, and its abundant irrigation water make it an ideal place to grow apples.

Now imagine an abandoned orchard with one gnarly old apple tree remaining, its limbs sagging under the weight of hundreds of tiny, knobby apples. Come next autumn, that same tree might bear few, if any, apples. That's because the tree, having depleted its energy stores, won't have enough reserves to make apple blossoms the following spring. This boom-or-bust cycle occurs naturally in apple trees.

Such erratic production would not allow a commercial grower to turn a profit—which is why 9 out of 10 orchardists thin their trees with chemicals, says Max W. Williams, research leader at the Production, Harvesting, and Handling of Tree Fruits Research unit in Wenatchee, Washington. Chemical sprays are far less expensive than the alternative, laborious hand thinning.

One such chemical, Elgetol, works by damaging the apple blossom's pistil, so the flower can't be pollinated. Treated trees set less, but larger, fruit.

But the procedure isn't risk free, says Williams. "Hot weather, for instance, can boost the chemical's action, causing accidental over-thinning," he says. Understanding the nuances of how such environmental factors affect the process is Williams' chief interest.

Different apple varieties respond differently to the thinning chemicals. Ethephon, another thinning chemical, can't be used on Red Delicious, for example, or it will lose its characteristic five-lobed shape.

Williams is currently cooperating with a grower near Wenatchee to see how some new varieties will respond to thinning chemicals. The new varieties, such as Fuji, Gala, and Jonagold, are increasing in popularity among growers because of a market glut in Red Delicious.

"So far, compared to other apples, Fuji doesn't respond as strongly to the chemicals, while Gala is more sensitive," says Williams. Eventually, he'll be able to recommend specific regimens to growers so they'll reap plentiful harvests with these newer varieties.

Coddling Enemies of the Codling Moth

As summer approaches and the tiny apples begin to grow, the fruit may fall victim to its worst enemy—the codling moth, *Cydia pomonella.*

In June, the grayish-white larvae of the small moths first burrow into golf-ball sized green apples ("codling" means small immature apple). The wormlike larvae feed on the apple's seeds and core, then tunnel out after they fatten up. Later, if these apples make it to the packing plant, workers on the sorting line toss them out.

Spraying orchards with the pesticide azinphosmethyl controls the worms. But recent studies in some Washington and California orchards show the pests are becoming resistant to the chemical.

The pesticide also kills several beneficial species—microscopic wasps and lady beetles that are natural enemies of insects that attack apples and apple trees. These pests—several types of aphids and tentiform leafminers—can damage apple tree leaves and shoots if their natural predators disappear.

Concerns over pesticide residues—both on fruit and in the environment—have increased interest in biological control methods.

Entomologists and chemists in Yakima's Fruit and Vegetable Insect Research Laboratory joined forces to test and improve a tactic known as mating disruption to stifle codling moths' sex lives.

Male codling moths, like many other insects, find mates by following an alluring chemical attractant, or pheromone, emitted by female moths. But orchards filled with synthetic pheromones leave males confused, and thus unlucky in love.

A 7-inch-long, one-quarter-inch-wide polyethylene plastic tube filled with synthetic pheromone slowly releases the attractants. To permeate an orchard, researchers twist five dispensers on an upper branch of each tree. Trees on the borders receive double the number of dispensers for extra protection against outside invaders.

To work effectively, the dispensers should be shaded by the leaf canopy, says chemist David F. Brown. "Sunlight rearranges the chemical structure of codlemone, the main component in the pheromone."

Brown, along with chemist Leslie M. McDonough and entomologist Harry G. Davis, continues to fine-tune the ratios of the pheromone components and study how temperature and time affect the dispenser's performance.

"We've gotten 85 to 90 percent control of codling moths in our test plots," says J. Franklin Howell, who conducts the field research. The plots were three-quarters of an acre or 1 acre in size, with 150 to 210 trees.

Even better, the researchers typically found larval damage in 1 percent or less of the apples from their experimental plots. That's the maximum level most commercial growers will accept, says Howell.

If the U.S. Environmental Protection Agency (EPA) approves the pheromone dispenser, large-scale testing of mating disruption can begin in commercial orchards, says James L. Krysan, research leader at the Yakima lab. "Organic growers are just itching to get their hands on the dispenser," adds Howell.

DOUG WILSON

Codling moth larva feeds on a Red Delicious apple. (K-3847-4)

All-Out Attack on the Apple Ermine Moth

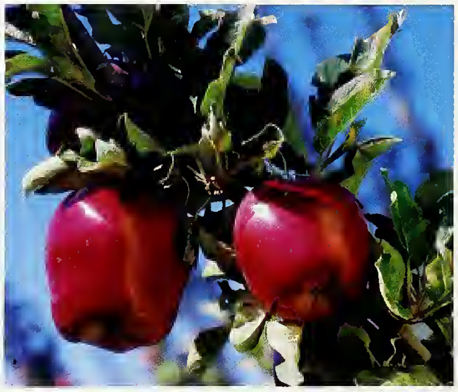
Another pest, the apple ermine moth *Yponomenta mallimellus*, also concerns Washington's apple growers. When the larvae emerge from tiny egg cases hidden on twigs, they devour apple tree leaves.

The moths, native to Europe and Asia, first appeared in Canada in the early 1980's. Moving southward, the moths invaded orchards in western Washington within 5 years.

Entomologists worried that central Washington would be next. McDonough and Davis developed a pheromone lure trap that helps in tracing the moth's spread, giving growers an early-warning system against the moth.

To make the lure, the scientists collected pest larvae from an infested tree, reared the insects, and snipped the pheromone glands from the female moths' abdomens.

DOUG WILSON



Red Delicous apples in an orchard near Wenatchee, Washington. (K-3853-5)

They determined the composition of the pheromone with laboratory instruments that reveal the chemical makeup of substances and with an electroantennogram, or EAG. The EAG uses tiny microelectrodes that are hooked up to the male's antenna (the moths detect the attractants with the hairlike projections).

After carefully taping a moth to a cork stopper, a researcher puffs various concentrations of the pheromone chemicals over a moth's antennae. The electrodes measure the moth's response to each scent. Results from tests revealed that two chemicals, in a ratio of 100:1, elicited the best response.

In the field test, thimble-size rubber stoppers were permeated with

The Apple Collection

In Geneva, New York, Rambo is an apple.

Rambo is one of more than 3,000 apples in the National Germplasm Repository that the ARS maintains in Geneva.

"It was a popular apple in the early 1800's " says ARS curator Philip L. Forsline.

But Rambo has faded from orchards and is preserved only in places like the repository, against the day when Rambo's genes could be needed in a breeding program to meet some future problem, he says.

Other historical varieties in the collection include the Lady apple—which was grown in 16th century France—and Esopus Spitzenberg, an apple that Thomas Jefferson is said to have admired.

And the collection includes the all-time great pie apple, Northern Spy, discovered in the early 1800's in a Mr. Chapin's seedling orchard only 15 miles from where the laboratory is located today. Northern Spy is still one of the country's premium processing apples.

From more modern times, the collection includes Freedom, developed and released just 7 years ago by the New York State Agricultural Experiment Station, where the germplasm repository is located.

Freedom is resistant to diseases such as scab, fire blight, and mildew and requires less pesticides . Genes for this disease resistance come from a wild apple found in Japan and preserved in the collection.

"The repository here often acts as a last ditch preservation for apples that would disappear because they've gone out of fashion," Forsline says. "Old varieties are vulnerable to the bulldozer as breeders retire.

"We've rescued a number of breeders' collections to preserve the genes," he adds. "But we're low on representatives of wild apples."

The ARS collection mostly contains *Malus x domestica*—commonly considered the domestic apple, which represents about 4 of the 30 species known worldwide.

But domestic apples have a narrow genetic base, having been selected for large fruit and not disease and insect resistance, Forsline says.

Wild apples have an amazingly wide range. They can be found growing from Siberia to France and all across the United States.

The repository is in the process of adding apples from Asia to fill in some of the gaps in the collection.

"Apples probably originated somewhere around the Caucasus mountains or north-central China, so we would especially like some specimens from those regions," says ARS geneticist Stephen Kresovich. "That's where the action will be for genes to improve apples here."

In 1989, an ARS team collected germplasm north of the border between the Soviet Union and China, including a city called Alma-Ata, which means "father of apple" in Kazakh, the native language of the region.

DOUG WILSON

Entomologist Franklin Howell attaches plastic pheromone dispenser to the branch of an apple tree. (K-3845-6)

the pheromones and suspended over a small cardboard trap coated with a sticky substance. Male moths homed into the lure, then fell and stuck in the traps, which were hung in trees.

"When we hung the baited traps in an orchard, we immediately got a good catch," says McDonough. In fact, the synthetic pheromone trap enticed even more moths than did three females held in tiny cages above the trap.

Traps set throughout Washington by the Washington State Department of Agriculture showed that the moths have indeed colonized central Washington near Wenatchee and George, where many of the commercial orchards are located. Monitoring the infestations will alert growers when to apply insecticides.

On a second front, a team led by geneticist Thomas R. Unruh, in Yakima, is already developing

CALVIN SPERLING

Rambo—a popular apple in the early 1800's.

Zhen-long Yan, a professor of botany from the Botanic Garden Academoa Sinica, Beijing, last year joined the Geneva staff on a temporary appointment and brought some Chinese species with her for the collection.

"She grew up and trained in botany in northern China. Her expertise is invaluable with the material we have and hope to have from that region," Kresovich says.

The sharing of apple germplasm works both ways. Yan has already sent some North American domestic and wild apples back to China.

The Chinese would like to expand the area in which they grow apples commercially. "Currently, most production is near the seashore. To grow them elsewhere in China will require apples that will do well in drier areas and where the soil is more alkaline," she says.

As the apple collection grows, it becomes harder to find land and labor to maintain it.

But Forsline and some colleagues may have the answer.

ARS scientists at the National Seed Storage Laboratory in Fort Collins, Colorado, working with Forsline, have developed a method to store dormant apple buds at sub-zero temperatures.

In 1988, a 25-year project was begun to study how to safely freeze apple buds in liquid nitrogen. Once frozen, buds will be removed yearly to determine whether they can still be restored to natural growth.

The buds are taken from winter dormant trees, then dehydrated and stored in liquid nitrogen at –196°C.

At just what point during winter dormancy it is best to take the buds has been a major question. Forsline tried harvesting buds early in December, after the coldest day of the winter in January, and in late February before spring growth began.

"We found taking the buds in December before the physiological need for winter rest is satisfied results in a better survival," Forsline says.

So far, results have been varied—100 percent viability of some varieties, as low as 10 percent of others. On the average, about 50 percent of the buds survive.

"Some varieties just take to cryogenic storage better," Forsline says. "A cold-hardy crabapple from Siberia did well."

If freezing proves a success, it will significantly cut down on the cost of storing each accession and allow expansion of the germplasm collection on much the same budget.

"We try to keep four of each type, two on dwarf rootstock and two on full-sized rootstock. But, the current budget doesn't allow it. Storing buds in liquid nitrogen would allow us to back up what we are growing in the orchard."—by **J. Kim Kaplan,** ARS.

Stephen Kresovich, Philip Forsline, and Zhen-long Yan are at the USDA-ARS Plant Genetic Resources Unit, NYS Agricultural Experiment Station, P.O. Box 462 Geneva, NY 14456 (315) 787-2244.

biological control methods to fight the ermine moth. Researchers imported more than 50,000 tiny wasps, *Ageniaspis fuscicollis*, from Europe and Asia in cooperation with scientists in Newark, Delaware; Paris, France; and Seoul, Korea.

The wasps, which are smaller than the dot on this i, find the camouflaged egg cases and lay an egg inside the moths' eggs. When the parasitized moth larva is nearly mature, each wasp egg splits numerous times by a process known as polyembryony. The phenomenon resembles what happens when a human embryo divides to form identical twins, except that the eggs continue dividing until about 100 eggs form—which eventually kills the moth larvae. Wasps have been released at sites in coastal northwest Washington and in the San Juan Islands of Seattle's Puget Sound each summer since 1988.

"The plan was to establish populations of these beneficial parasites in western regions where few apples are grown commercially," says Unruh. "Then we'd use these reservoirs against the ermine moth when it appeared in commercial orchards.

The scientists recently recovered populations of two races of *Ageniaspis* near Bellingham, Washington, that had been released in July 1989—proof that colonies of the beneficials were successfully established.

DOUG WILSON

Excess oxygen during storage triggers scald, an unsightly browning of apple skin. (K-3851-8)

To build an even stronger arsenal, Unruh plans to rear other species of parasitic wasps that could help squelch invading moths in the field.

Mechanizing Apple Harvest

Since harvesting can be as much as 25 percent of total production costs, some scientists feel that mechanical harvesting holds a key to making apple production more profitable.

Stephen S. Miller, head of ARS' Appalachian Fruit Research Station in Kearneysville, West Virginia, feels that research on mechanical harvesting should be a top priority.

He notes that machine picking has generally been unsuccessful in the past because of bruises and other damage apples sustain during the operation. A way around this, he believes, is to change the basic shape of the apple tree and to engineer the machines to fit that shape.

"Tree training," Miller says, "is the most important cultural practice in developing an orchard." Size, form, and canopy density ensure a manageable and efficient fruiting tree. Also, there must be enough space under the tree to allow movement of equipment during harvest.

Miller and ARS engineer Donald L. Peterson at Kearneysville are perfecting an over-the-row harvester. Since tests with mechanical harvesters began in 1983, more fruit picked from smaller, spur-type trees has qualified for top grades, fancy and extra fancy, than fruit picked from larger trees and nonspur trees.

Biocontrol—Beyond Bugs

Apples that survive the orchard unscathed by insects still aren't in the clear. Harvested apples, placed into wooden crates and dragged over soil, are often exposed to microorganisms that cause mold and rot.

Mucor rot turns an apple's flesh brown, soft, and watery; the molds spread whitish, blue, or gray fuzz over the fruit's skin.

Treating fruit with fungicides controls some of this decay, but naturally occurring yeasts may provide a safer option.

Rodney G. Roberts and coworkers at the Wenatchee lab screened more than 175 strains of yeasts and bacteria. They discovered four *Cryptococcus* strains that inhibit the molds and rots.

These beneficial yeasts, which were found on the leaves and fruit of apples and pears, work in still-mysterious ways.

"Some researchers thought the yeasts used up the sugars that the

molds needed for growth or that they made an antibiotic that killed the fungi," says Roberts. But their results haven't supported those theories, he adds.

While trying to solve that mystery, Roberts is also investigating ways to produce and store the yeasts on a large scale suitable for commercial growers. He's found that the yeasts work well even in the special low-oxygen refrigerated units used to store apples.

That's good news, since as much as 70 percent of a year's apple crop can end up in storage. These usually end up in controlled atmosphere, or CA, storage.

Better Ways To Store Apples

During the long, dark months of winter storage, flavor essences that give apples their characteristic sweet-tart flavor may slowly seep out.

Plant physiologist James P. Mattheis studies how and why different storage conditions affect apple taste. In glass jars, he traps the volatiles that escape from fruit taken from CA storage. These chemicals are then condensed, concentrated, and injected into a gas chromatograph, a machine that can quantify minute amounts of chemicals.

When apples are stored with very little oxygen, they stop producing some of their volatiles, says Mattheis. Tests are now under way to see if increasing oxygen levels after a few months of storage will stimulate fruit to make flavor components again. Too much oxygen, however, can make the fruit mushy, so it's a tricky process.

Yet another problem with excess oxygen during storage is unsightly, brown skin patches on apples, known as scald. The condition is similar to what happens to a freshly cut apple: The presence of oxygen triggers browning.

Ethephon, the agent used to thin trees, also cuts down on scald when sprayed on Granny Smith and Fuji apples 2 to 4 weeks before harvesting, says Eric A. Curry, another plant physiologist at Wenatchee.

Giving apples a nice warm bath before their chilly storage also helps reduce scald, a method first discovered 25 years ago. To determine how hot and how long to make the bath,

DOUG WILSON

Gray mold turns an apple's flesh brown and soft. Scientists at the Wenatchee Lab have discovered four beneficial yeasts that inhibit such diseases. (K-3852-11)

Curry's now experimenting with Red Delicious apples.

"This technique could cut down amounts of antioxidant chemicals that are now used to control scald," he contends.

So could better CA storage methods—like super-low oxygen levels for the first few days of storage. Curry and colleague Steven R. Drake are currently testing Red Delicious varieties.

Such tests should be simpler, thanks to a state-of-the-art, computerized CA storage system now available at the Wenatchee lab. Two walk-in coolers contain 30 separate chambers that can each hold 2 bushels of apples. A few quick keystrokes on the computer will automatically set the amount of oxygen and carbon dioxide in each chamber.

"We'll be able to do many different manipulations in just one season, instead of taking several years," says Drake. The added chambers allow the researchers to run multiple repetitions of the different regimens.

Recent studies showed that dropping the oxygen levels within 24 hours after the apples go into storage, as opposed to the typical week or 10 days, makes apples more tasty. The process, known as CA shock, slows the fruit's metabolism even more than regular CA, so growers could leave apples hanging on the tree longer to develop more flavor.

After apples come out of storage, there's no harm in attending to their appearance. But the glossy finish on apples in supermarkets does more than attract buyers. It also helps conserve the apple's color and crispness. Waxes, says Drake, act like "a mini-controlled atmosphere," to keep apples fresh. He's determined better ways to apply the coatings, which are made from natural compounds like carnauba, a wax from a Brazilian palm tree.—By **Julie Corliss,** ARS. **Doris Stanley,** ARS, contributed to this article.

Max W. Williams, Eric A. Curry, James P. Mattheis, Stephen R. Drake, and Rodney G. Roberts are at the USDA-ARS Production, Harvesting, and Handling of Tree Fruits Research Unit, Wenatchee, WA, 98801 (509) 664-2280; David F. Brown, Harry G. Davis, J. Franklin Howell, Leslie M. McDonough, Thomas R. Unruh, and James L. Krysan are at the USDA-ARS Fruit and Vegetable Insect Research Unit, Yakima, WA 98902 (509) 575-5900; Stephen S. Miller is at the USDA-ARS Appalachian Fruit Research Station, Rte. 2, Box 45, Kearneysville, WV 25430 (304) 725-3451. ◆

Of Crops and Crawfish: Diversity Sweeps the South

For Mississippi Delta farmers, making a living is rarely easy—especially for the small farmer.

But horticulturist Xenia Y. Wolff, working at the ARS Soil and Water Research Laboratory in Baton Rouge, is trying to make things a little easier.

Now in her second year of a 2-year appointment to work at the Center for Small Farm Research at Southern University and A & M College in Baton Rouge, Wolff, together with Owusu Bandele, a professor of horticulture at Southern University, is looking for new crops and cropping systems that could provide small farmers in the Delta with different and profitable harvests.

"The small farmers in the Delta and surrounding region need alternatives to the traditional Delta crops so they can diversify," Wolff explains. "In particular, our farmers need low input crops that will return a good profit on small acreages without major capital investments."

Wolff and Bandele have cast a wide net for alternative crops. "There isn't just one crop that will be the answer. Each new crop means a few more farmers could add a cash producer," Wolff says.

The possible answers they have come up with so far range from an ancient crop of the Incas called goldenberry to raising dill for the local crawfish-processing industry.

Bandele had found that crawfish processors, who require large amounts of fresh dill for products for the European market, were unable to find local sources. Dill is not commercially grown in the Delta region.

"The processors I talked to were often unhappy with the quality of the dill as it arrived after being shipped in from California and other distant locations. The idea of being able to buy fresh dill from local sources sounded great to some of them," Bandele says.

KSM, a crawfish -processing plant in East Baton Rouge Parish, says it uses about 150,000 pounds of fresh dill and 30,000 pounds of dill seed during crawfish season and would be happy to buy locally produced dill if it was of good quality. And KSM is only one of numerous processors in the region.

Traditionally, dill is a cool-climate crop. Wolff and Bandele are working out how to best cultivate it in the Delta climate.

"Different growing regimes with different fertilizer amounts and schedules, planting densities, and variety choices can give you dill with different levels of carvone, the compound that gives dill its characteristic aroma and flavor," Wolff says. "The idea is to grow dill with the highest amount of carvone, since crawfish processors generally prefer very aromatic dill."

PERRY A. RECH

Using a refractometer, horticulturalist Xenia Wolff tests for sugars derived from muscadine grapes. (K-3820-7)

Dollars From the Goldenberry

Another possible new crop for the Delta that Wolff is working on is actually an ancient one called the goldenberry.

The goldenberry, also called cape gooseberry or a ground cherry, although it is neither a true gooseberry nor cherry, has been a minor crop of the Andes since ancient times where it was grown by the Incas. Today, goldenberries are also grown in Australia, New Zealand, Africa, California, and Hawaii where they have given rise to a specialty market as a preserve under the Hawaiian name "poha." Europeans often pay premium prices for goldenberries dipped in chocolate or as decorations on cakes and tortes.

There is also growing interest in goldenberries as an item for upscale restaurant menus.

"It could be the next kiwifruit," Wolff says, referring to the multimillion dollar market that has developed since the 1960's for the once unknown kiwifruit. "It can even be dried as a raisin."

For the Delta farmer, goldenberry offers a highly productive, low-input crop. "They produce like crazy," Wolff says. "We had several harvests of one-half to a pound of fruit per plant each time."

The berries, about the size and texture of a cherry, ripen to a bright yellow inside a papery husk shaped like a Chinese lantern. "The taste is tart—somewhere between a plum and a tomato, although some people think it has a nutty taste like a pecan," Wolff says.

Goldenberries will grow almost anywhere tomatoes—to which they are distantly related—do. However, goldenberries are very hardy and do well on poor soil. Extensive fertilizing only encourages vegetative growth and hurts berry production.

The seeds are somewhat delicate to germinate. "I've found sprinkling them on moist potting soil and covering them with plastic wrap creates a greenhouse environment that helps them germinate," Wolff says.

Wolff is still working out the best cultivation methods. She is also experimenting with variety selection, looking for the one that will do best under local conditions. She has nine different varieties, some upright and bushy, others prostrate and viny.

So far, no major insect or disease problems have shown up that would make the Delta region inhospitable for the goldenberry.

"But the goldenberry's real success will probably depend more on marketing and advertising than it will on farmers' ability to grow them," she says.

PERRY A. RECH

Goldenberries, about the size and texture of a cherry, ripen to a bright yellow inside a papery husk. (K-3819-5)

Vegetable Pears

Chayote, a white or green pear-shaped relative of the cucumber known locally as a mirliton or vegetable pear, also looks like a potential commercial crop for the region, according to Wolff. It is grown in many Louisiana backyards, but commercial production is very small.

Wolff and Bandele are investigating the best way to commercially cultivate this vine crop, including fertilizer, lime, and water requirements. "It is very sensitive to too little or too much water," she says. "In the greenhouse, it needed about 1 inch per week, but that is within the potential of the region."

Too much rain is more likely than too little to be a problem in the Delta, so Wolff recommends that the soil be lightened for better drainage with peat moss and animal manure, which also adds nutrients.

"In my greenhouse study, chayotes did the best when they were fertilized with rabbit manure. Some small farmers are raising rabbits around here, so they could be recycling the rabbit manure into the fields," she says.

In addition to completely alternative crops, Wolff has been working out ways that Delta grape and berry growers can produce cash crops while they wait for the primary crop to reach maturity 3 to 5 years after planting.

Muscadine grapes do well in the Delta area, but growers have a cash-flow problem during the 3 to 5 years before vines produce major harvests.

"The question was what could you intercrop among the vines that would give the grower early cash returns without adversely affecting growth and development of the grapes," Wolff says.

Wolff and Bandele have begun experiments to compare yields of intercropped and monocropped grape vines. The results will include comparing the economic returns of the two systems.

Broccoli, cucumber, and okra have all done well as intercrops, although analysis of yield has not yet been completed.

"As a matter of fact, the grapes in the intercropped plots appear more vigorous and fruitful than those planted without vegetable intercrops," Bandele says. "This may be a result of increased drainage due to cultivation between the rows, fertilizing differences, allelopathy, or a combination of these factors."

With rabbiteye blueberries, which need acid soil and have a 3-year wait for maturity, successful intercropping calls for snapbeans, okra, or cucumbers.

"Blueberries are planted with about 12 feet between rows anyway, so there is lots of room for intercropping, especially while the berry plants are young," Wolff says. "You can get two rows of cucumbers in that space."

Wolff has already established that she can get good yields from her intercropping harvests. But she is still calculating the potential economic return.—By **J. Kim Kaplan,** ARS.

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